

Tattooing as an individual marking technique in urodeles

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A review of the literature shows that no marking method of urodeles is entirely satisfactory. The choice of a method has to take into account the number of animals to be marked, the duration of the observations and the goals to be reached. Skin staining by tattooing is not often used even though such a technique gives interesting results. Tattooing is a quick procedure which makes it possible to mark a great number of animals individually. The remanence of the mark reaches at least three years. No harmful effects have been observed.

INTRODUCTION

Marking has always been a problem in studies of population dynamics in urodeles. No technique is entirely satisfactory and most often the solution chosen is a compromise.

Using rings, like bird bands, is made difficult because of the small size of urodeles limbs. Such rings involve a loss of mobility. We tried caudal rings derived from those applied to anuran limbs (DELY, 1954 ; NACE & MANDERS, 1982). Such rings, on which beads or symbols can be threaded, made it possible to study individual behaviour in the laboratory (MARTIN et al., 1989). But we sometimes observed newts trapped in a clump of vegetation, a blade of *Myriophyllum* wedged in the ring. After some time, necrosis may occur around the ring and may involve the loss of both a piece of the tail and the tag. The animal mobility would then be reduced until the lost part was regenerated.

Jaw tags used in anurans (RANEY, 1940) are too large for most urodeles and the risks of necrosis are the same as for caudal rings (RANEY, 1941).

Marking by branding avoids the need of carrying a foreign object and seems to be less traumatizing. Heat-branding using red-hot metal wire may make it possible to recognize a toad at least one year after marking (CLARK, 1971). But such a method has never been applied to urodeles. Freeze branding, using wires immersed in dry ice gives interesting results in fish (RICKER, 1956). In the anuran *Ascaphus trueti*, scars remain readable for at least two years (DAUGHERTY, 1976). In urodeles however, freeze-branding scars don't persist longer than a few weeks (GEIGER et al., 1982, NACE & MANDERS, 1982, and personal observations) and may be traumatizing (KLEWEN, 1982). It is probable that such a regeneration capacity of the derm also rules out the heat branding technique.

Marking by toe-clipping is the technique which has been most frequently used for individual marking. Formalized by MARTOF (1953) in the study of a green frog population (*Rana clamitans*), such a method has been used in urodeles in the studies of TWITTY and his co-workers about the homing capacities of *Taricha rivularis* (synthesis in TWITTY, 1966). If it makes it possible to recognize a great number of individuals, it nevertheless has three major disadvantages :

(1) Urodele toes regenerate quickly : sometimes total regeneration requires less than one year. HEATWOLE (1961) suggested applying beryllium nitrate to inhibit regeneration. But, besides the use of such a highly toxic chemical is dangerous, its efficiency seems to be limited to temperatures below 18°C (EFFORD & MATTHIAS, 1969).

(2) The amputation of more than two digits involves a loss of mobility in anurans. The probability of recapturing a marked individual is inversely related to the number of clipped toes (CLARKE, 1972). The same observation is to be expected in urodeles.

(3) Newts often show natural digit amputation in the field ; this may be due to predatory insects like dragonfly larvae, particularly benthic *Libellula* larvae, or to small bivalves, like *Sphaerium*, which are often found gripping a toe.

Recognition of an individual thanks to its coloration pattern means organizing a collection of photographs. That efficient method may however only be used in species in which a pattern of spots varies from one individual to another, as on the backs of *Salamandra salamandra* (JOLY, 1968) and of *Notophthalmus viridescens* (HEALY, 1974) or on the belly of *Triturus cristatus*, *T. vulgaris* (HAGSTRÖM, 1973) and *T. boscai* (DIAZ-PANIAGUA, 1986).

In other cases, RAFINSKI (1974, 1977) recommended marking by autotransplantation of a piece of ventral skin on the back and *vice-versa*. An Alpine newt (*Triturus alpestris*) may support ten such grafts. Despite the advantage of giving permanent marks, that method has two major drawbacks :

(1) it concerns only species with contrasted body colours ;

(2) the time needed to perform several grafts and the limited number of combinations are handicaps for the study of numerous populations.

Lastly there are the skin staining techniques. For marking toads, WISNIEWSKI et al. (1980) and GITTINS et al. (1980) suggested the injection of alcyan blue into the limbs by use of a high speed anaesthetic injector, usually used by odontologists (Panjet or Dermojet). Using such an injector we tried to mark the belly skin of Alpine newts. The speed of projection was so strong that the jet of dye crossed the animal's body and came out on the other side, but without involving the animal's death. We estimated however the risk of organ injury to be too high to accept that technique for urodele marking.

The intradermic injection of a dye may be made possible by scarification or tattooing, as applied in anurans by KAPLAN (1958, 1959) or by using a spray gun (NISHIKAWA & SERVICE, 1988). Marks remain readable for at least two years and the entire marking procedure takes only a few minutes. The aim of our paper is to describe testing of such a technique in the newts *Triturus alpestris* and *T. helveticus*.

METHOD

TECHNIQUE

Its principle is to draw a pattern of spots on the animal's belly. Each spot is drawn by tattooing using an electric tattooer ; such a device is usually used by veterinarians for marking pets or farm animals. Alcyan blue and Indian ink provided colouring.

The newt is first anaesthetized by phenoxyethanol. Drawing a spot involves applying the tattooer for about 20 s. In order to obtain a 2 mm spot, the needles must be shifted slightly several times.

After rinsing the surplus dye (a paint brush is useful), the spot must be uniformly coloured and stand out well. The tracks of the tattooer needles have to be invisible. If the design is not correct, the procedure has to be repeated.

CHOSEN PATTERNS

Marking according to a site

That coding procedure makes it possible to estimate the newt flow between several sites. One spot indicates the site where the first capture occurred. If the newt is caught again in another site, a second spot is drawn according to a marking code. In the study of a *Triturus helveticus* population inhabiting four neighbouring ponds, each pond corresponded to a spot situated near a limb. Two spots meant that the animal had been caught at least twice in two different ponds, which were identified. Newt flows were thus estimated by a quick marking procedure and the organization of a complex file is unnecessary. But it does not provide information about individual behaviour.

Individual marking

It requires drawing a higher number of spots. In the case of a population of *Triturus alpestris*, we used a maximum pattern of 11 points. They were placed according to three longitudinal rows, which are situated on the right side, the middle and the left side of the belly. Each row can contain 5 spots. The middle row indicates the site of the first capture. It had only one spot. Lateral rows are used for individual recognition. If the newt moved from one site to another, no new spot is necessary, the animal retaining the individual code. In the data file, the individual code consists of three numbers :

- the first corresponds to the site of first capture, and ranges from 0 to 5 ;
- the second and the third correspond to right and left rows respectively ; they are composed of 0 to 5 figures, each ranging from 0 to 5.

Fig. 1 gives an example of the reading of such a code. Assuming the observer is able to recognize sex, such a coding system makes it possible to identify 2048 individuals. With only four spots per row, 512 newts may be recognized.

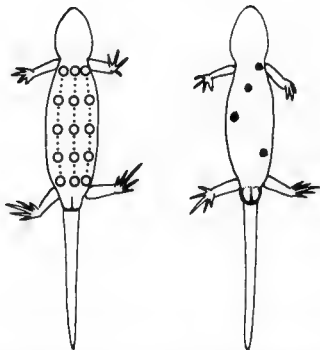


Fig. 1. - Marking patterns of newts. Left : overall view of the possible marking sites on the newt's belly : in the study of an Alpine newt population which was shared among 5 ponds, the two lateral rows were used for the individual marking whereas the middle row corresponded to the site of first capture. Right : example of a code : male newt n° 2/3/14 (a point at the second place on the middle row, a point at the third place on the right row, two points at the first and the fourth places on the left row).

RESULTS

PERMANENCE OF THE MARKS

In the laboratory, the suppression of hibernation, the high level of temperature (ranging from 16 to 25°C) and the abundance of food constitute conditions of frequent skin renewal. After two years under such conditions, marked Alpine newts could still be identified, despite a considerable decrease in the spot contrast. Under natural conditions, we suppose that the spot remains readable for at least 3 years.

INNOCUOUSNESS

In the laboratory, none of the 4 marked newts died during the first year following the marking procedure. One of them died after one year. We can attribute such a death to natural mortality because the animals were relatively old when marked.

Table I. - Relation between number of spots and recapture probability.

Spots number	N newts marked in 1987	% recapture in 1988
2	68	19
3	140	12
4	41	22

Estimating the impact of marking on a wild population is more difficult. One possible way may be to compare the probabilities of recapturing a newt according to the number of spots drawn on its belly. If the method is harmful, the probability of recapturing should be related to the number of drawn spots. But no clear relation appears between percentages of recapture between two successive years and the number of spots (Table I).

DISCUSSION

Electric tattooing exhibits some interesting advantages :

- it makes the recognition of a great number of individuals possible;
- the marking lasts longer than that provided by toe-clipping;
- numerous animals may be treated quickly;
- the method seems innocuous.

However it does not provide the life-long permanence of the autotransplantation method. But the time needed for individual marking restricts the use of the latter method to small populations. The choice of one or the other (when recording of body pattern is not possible) depends on the kind of problem being dealt with and on the chosen experimental design.

Such a technique may be used in the field with a portable generator to provide electricity.

Because of their ventral position, the spots drawn on the belly are not apparent when the animal shows a normal posture. One may suppose that they do not interfere with the visual signals acting during courtship behaviour.

The use of such a marking technique is restricted to adult animals, and the problem of marking young newts in order to estimate juvenile dispersion is still unsolved.

The tattooing technique could be improved by trying new dyes which would last longer or would increase the number of combinations by diversifying the colours used.

Useful address : we used an electric tattooing device, which may be found at Veto-équipement , 188 avenue Roger Salengro, 69120, Vaulx-en-Velin, France.

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